THE PARATHYROIDS

BASIC AND CLINICAL CONCEPTS

THIRD EDITION

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Preface to the Third Edition

This year marks the twentieth since the first edition of *The Parathyroids* was published in 1994. That book was a milestone because a book dwelling exclusively on the parathyroid glands and their associated disorders had not been published since the classic text *The Parathyroid Glands and Metabolic Disease* of Albright and Reifenstein in 1948. What Albright and Reifenstein's remarkable book established cannot be underestimated, and historically we look back and marvel at the insights and foresights it contained.

The first edition of *The Parathyroids* was a fitting sequel and literary heir to the Albright and Reifenstein book, albeit coming more than four decades later! In the intervening 46 years, the field advanced at a remarkable pace, as the modern tools of biomedical research revealed exciting and unanticipated insights into the fundamental principles of calcium homeostasis, parathyroid hormone, and disorders related to the parathyroid glands. The second edition of *The Parathyroids* followed eight years later, in 2002, with key updates on the structure and function of the parathyroid hormone gene and parathyroid hormone itself, along with new knowledge of the regulatory control of parathyroid hormone synthesis and secretion. Knowledge of primary hyperparathyroidism, the classic disorder of parathyroid hyperfunction, was updated to describe a clinical spectrum of presentations and a summary of management guidelines. Advances in molecular genetics called attention to familial syndromes of primary hyperparathyroidism. The second edition included the hypoparathyroid disorders and a section on the development of parathyroid hormone as a therapeutic treatment for osteoporosis.

Twelve years later, the parathyroid field has continued to move rapidly forward with new insights into basic, translational, and clinical aspects of parathyroid functions and parathyroid hormone. *The Parathyroids*, third edition, is clearly needed at this time. As was the case for the previous two editions, this book is intended for students, teachers, practitioners, and investigators.

To accommodate the major advances over these dozen years, we reorganized the book. The first two sections of the second edition are now contained in a single section entitled: *Molecular*, *Cellular*, *and Physiologic Aspects of the Parathyroids.* The chapters in this section now focus more distinctly upon PTH or PTHrP rather than the combined approach taken in the former edition. This reorganization results from our greater appreciation of the separate and distinct roles served by PTH and PTHrP in cellular, molecular, and biochemical terms. In Section II, 20 chapters cover *Clinical Aspects of Primary Hyperparathyroidism* with new chapters on epidemiology, normocalcemic primary hyperparathyroidism, primary hyperparathyroidism in children, non-traditional aspects of primary hyperparathyroidism, and vitamin D in primary hyperparathyroidism. Section III, *Non-parathyroid Hypercalcemic States*, updates the reader on the differential diagnosis and management of hypercalcemic states, other than those directly related to PTH. The hypercalcemias associated with PTHrP, local and ectopic syndromes, and genetic disorders are covered. Section IV, *Secondary and Tertiary Hyperparathyroid States*, has been expanded to include more extensive discussion of the interrelationships between vitamin D and parathyroid hormone, as well as a separate chapter on the causes of secondary hyperparathyroidism, beyond those related to renal disease and vitamin D deficiency.

Over the past decade, interest in hypoparathyroidism has intensified with new insights into etiology, epidemiology, molecular genetics, and therapeutics. Accordingly, Section V, *The Hypoparathyroid States,* has doubled in size with six new chapters on epidemiology, etiology, clinical presentations, skeletal involvement, and treatment. Similarly, Section VI, *The Parathyroids and Osteoporosis*, provides new information on monotherapy and combined therapy with parathyroid hormone, and on the potential expansion of parathyroid hormone therapeutics to include fracture healing.

The enormous scientific advances of the past twelve years mandated not only a complete reorganization of the book but also provided an opportunity to welcome new Associate Editors to our team. The original team of Bilezikian, Marcus, and Levine has been expanded to include John Potts, Claudio Marcocci, and Shonni Silverberg. We have endeavored at all times to provide the right balance of editorial oversight to our authors. With the passage of time, it also became clear that many chapters, both the new and the revised ones, needed input from those who have made the most important contributions over the past decade. We are pleased to welcome 35 new principal authors to the book while thanking their predecessors for a job well done.

A generation of life—and several generations of medical progress—have passed since the first edition of *The Parathyroids*. Many of you undoubtedly never had the great privilege to know or to train with Gerald D. Aurbach to whom *The Parathyroids* was initially dedicated. His untimely and tragic death in 1991 was our catalyst and inspiration for this book. We remembered Jerry then for his "wisdom, scientific acumen, investigative skills, and daring insights." We remember him now, so many years later, in much the same way. We remain mindful of the role Jerry had not only in our careers but also for the entire field, which he helped to create. His scientific progeny now have entered the stage of "grand progeny" if not "great grand progeny." Notwithstanding the passage of time, the imprint of Jerry's influence remains great. This book honors Jerry's legacy and we are proud to continue to remember him for what he meant to us and the entire field.

We wish to thank Mara Conner, Jeffrey Rossetti, and Megan Wickline of Academic Press, Elsevier, for their tireless and collegial efforts at all times in the making of *The Parathyroids, Third Edition.*

Enjoy the book.

John P. Bilezikian
Robert Marcus
Michael A. Levine
Claudio Marcocci
John T. Potts
Shonni J. Silverberg

Preface to the Second Edition

The first edition of *The Parathyroids* was published in 1994. It marked a milestone in the field, carrying on the tradition of Albright and Reifenstein whose 1948 classic *The Parathyroid Glands and Metabolic Disease* established a key role for the parathyroids in calcium homeostasis and metabolic bone disease. In *The Parathyroids,* we assembled a body of knowledge that had been accumulating over a 30-year period. The spectacular pace of discovery placed the tiny parathyroid glands at an epicenter of an enormous research effort in metabolic bone disease. The first edition was used widely and filled an essential gap in reference literature. Over the past seven years, as this field has continued to grow, with newer and greater appreciation of the role of the parathyroids in the overall governance of calcium homeostasis, a second edition appears to be particularly apt.

The second edition of *The Parathyroids* contains chapters that have been extensively revised and expanded and many new chapters as well. The chapters document our new knowledge about virtually every facet of this field and reexamine classic precepts that have stood the test of time. We understand better than ever before the structure and function of the parathyroid hormone gene and protein as well as the regulatory control of parathyroid hormone synthesis and secretion, the physiological and pathophysiological aspects of parathyroid hormone-related protein (PTHrP), the mechanisms of parathyroid hormone and PTHrP action, and the cell biology of PTH and PTHrP. With regard to primary hyperparathyroidism, we now appreciate a spectrum of clinical presentations according to where in the world it is detected. Information about the course of primary hyperparathyroidism with and without parathyroid surgery is also new, as are the molecular genetics, biochemical, and histomorphometric dynamics of primary hyperparathyroidism. Advances in preoperative localization of parathyroid tissue and newer operative approaches to parathyroid gland surgery are noteworthy. The hypoparathyroid disorders are understood better with regard to their molecular genetics, pathophysiology, and mechanism. Finally, newer information is available about how parathyroid hormone can be both a catabolic and anabolic hormone for bone. This newer knowledge has fueled provocative ideas about the pathophysiology of osteoporosis and is heralding a new era in the therapeutics of osteoporosis. The second edition, thus, is still a comprehensive examination of basic and clinical concepts of the parathyroids. It is intended for students, teachers, practitioners, and investigators.

In light of these newer developments in the field, the second edition has been reorganized to provide the reader with information that follows best the changing scientific logic. Fifty-five chapters are divided into seven sections. In Section I, nine chapters are devoted to basic concepts of parathyroid hormone and PTHrP, covering embryology, anatomy, and pathology of parathyroid tissue; gene structure, biosynthesis, and metabolism of PTH and PTHrP; receptors, nuclear targeting, and signal transduction for PTH, PTHrP, and calcium ion; and a comprehensive review of the immunoassays for PTH and PTHrP. In Section II, eight chapters are devoted to the physiological aspects of calcium metabolism and the anabolic and catabolic effects of PTH at the level of bone and bone cells. Five chapters cover in detail all aspects of PTH and PTHrP with regard to traditional and nontraditional target organs. In Section III, 21 chapters are devoted to clinical aspects of primary hyperparathyroidism. Chapters on the growth of normal and abnormal parathyroid cells and the molecular genetics of primary hyperparathyroidism are followed by three chapters that describe different clinical presentations of primary hyperparathyroidism throughout the world. Detailed coverage of bone dynamics and stone disease is followed by information relevant to the medical and surgical management of primary hyperparathyroidism. Also covered are other presentations of primary hyperparathyroidism: as a malignancy, as an acutely hypercalcemic disorder, and in association with the multiple endocrine syndromes I and II. A chapter on familial hypocalciuric hypercalcemia completes this section. Two chapters in Section IV cover the parathyroids in renal disease. These are followed by six chapters in Section V that focus on special considerations. The first three chapters review the differential diagnosis of hypercalcemia, including syndromes caused by the local and systemic production of hypercalcemic factors such as PTHrP. Jansen's disease, the acute management of hypercalcemia, and hypercalcemia in children are considered in separate chapters. In Section VI, the hypoparathyroid states are reviewed in six chapters, which cover molecular, ionic, and immunological defects in the hypoparathyroid states and the role of hypoparathyroidism in the differential diagnosis

of hypocalcemia. In Section VII, the role of parathyroid function in osteoporosis is covered in three chapters describing changes in parathyroid function with aging, parathyroid function and responsiveness in osteoporosis, and the potential of parathyroid hormone as a therapy for osteoporosis.

As was true for the first edition, we recognize that this book is not likely to be read from cover to cover. Thus, each chapter has been written to provide a body of knowledge that can stand alone. The chapters, however, are also liberally cross-referenced to help the reader continue reading more directly related material if desired.

The first edition of this book was dedicated to the memory of Gerald D. Aurbach, whose untimely and tragic death was its catalyst and inspiration. Virtually all the principal authors of the first edition had known and worked with Jerry. We remembered him then for his "wisdom, scientific acumen, investigative skills, and daring insights." We remember him now in much the same way. We were and still are mindful of the role Jerry had not only for us but also for the entire field, which he helped to create. We were his scientific progeny. It is 10 years since Jerry's death, virtually a generation in the world of science. As a result, some of the leading figures in this field have emerged without having had the special privilege of working with or knowing Jerry. The authorship of the second edition has been broadened, therefore, to include the very best in our field, recognizing that although Jerry's legacy is still alive, it now extends to an even broader cross-section of the field.

We wish to thank Jasna Markovac of Academic Press, who was instrumental in both the first and current editions of *The Parathyroids*. Mica Haley of Academic Press was also most helpful in attending to the many details required to ensure a rapid turnaround time to final publication.

Enjoy the book.

John P. Bilezikian
Robert Marcus
Michael A. Levine

Preface to the First Edition

One of us (JPB), dreamed of this book about five years ago. It seemed then that advances in our knowledge of the parathyroids represented nothing less than a 30-year revolution of spectacular progress. We gained knowledge over this period at an explosive pace with a concomitant new appreciation of the basic and clinical ramifications of these four tiny endocrine glands. The major secretory product, parathyroid hormone (PTH), was isolated, sequenced, assayed, and cloned. PTH became one of the first hormones to be shown to utilize cAMP as a second messenger. Regulation of PTH synthesis and secretion by calcium and 1,25-dihydroxyvitamin D was appreciated, as well as the cellular effects of PTH on its two major target organs, bone and kidney. The discovery of parathyroid hormone-related protein (PTHrP) as a cause of hypercalcemia of malignancy and a more general appreciation of PTHrP and PTH as polypurpose factors with many diverse biological effects represent exciting new advances in our field. The recent cloning of a bona fide receptor for both PTH and PTHrP is a tremendous achievement, as is the thinking that both PTH and PTHrP may utilize more than one second messenger pathway, and perhaps interact with more than one receptor. At the clinical level, we have seen a remarkable evolution in the presentation of primary hyperparathyroidism and are beginning to understand molecular features of this disease. Pseudohypoparathyroidism is now appreciated, in its classical form, to be a G-protein deficiency disease. Autoimmune and molecular features of hypoparathyroidism have been identified and studied. New knowledge of the pathophysiology of secondary hyperparathyroidism associated with renal failure has had a direct impact on management and clinical outcome. PTH is now appreciated to have important anabolic properties in bone that may have implications for its use as a therapeutic agent in osteoporosis. This incomplete summary argues persuasively for how fast and how far this field has advanced.

This is not to say that we were in the dark ages before Aurbach isolated parathyroid hormone. Certainly, it was Fuller Albright who in 1948 correctly pointed out that "back in the dark ages of endocrinology, in the early 1920s, hyperparathyroidism was an unknown fact." It was also Albright who reminded us of the work of Sandstrom, who in 1880, 40 years before the first known cases of hyperparathyroidism wrote, "The existence of a hitherto unknown gland in animals that have so often been a subject of anatomical examination called for a thorough approach to the region around the thyroid gland even in man. Although the probability of finding something hitherto unrecognized seemed so small that it was exclusively with the purpose of completing the investigations rather than with the hope of finding something new that I began a careful examination of this region, so much the greater was my astonishment therefore when in the first individual I examined, I found on both sides at the inferior border of the thyroid gland an organ of the size of a small pea, which judging from its exterior, did not appear to be a lymph gland, or an accessory thyroid gland, and upon histological examination showed a rather peculiar structure."

The first chapters on the parathyroids were indeed written by Albright and a band of spectacular clinical investigators of the 1920s, 1930s, and 1940s. These chapters are recorded in the Albright and Reifenstein classic *The Parathyroid Glands and Metabolic Disease.* We recommend this insightful 45-year-old book as important and provocative reading. *The Parathyroids* is designed to follow the Albright and Reifenstein text. Certainly all endocrinology reference texts routinely include a section on the subject matter of this book. Other texts that are more focused on calcium metabolism provide more information than the standard endocrinology texts on the parathyroids. However, there is no book that is exclusively devoted to a comprehensive examination of basic and clinical concepts of the parathyroids. As indicated by the size and scope of *The Parathyroids,* it is clear that a book devoted to this subject is worthy and long overdue. It is time for such a book to stand on the endocrine shelf near its anatomical partner, the thyroid gland, which in Werner and Ingbar's *The Thyroid* has had its own literary repository since 1955.

This book is intended for students, teachers, practitioners, and investigators of this field. It covers in a current and concise yet complete manner virtually all that we know about the parathyroids. Thus, it is both a basic and a clinical text. The 51 chapters are divided into a presentation of basic knowledge of the parathyroids and the clinical disorders associated with dysfunction of these glands. Section I, Basic Concepts of the Parathyroids, consists of 22 chapters. Chapters 1–7 cover the embryology, anatomy, and pathology of the parathyroid

glands; calcium homeostasis; regulation of parathyroid hormone by dietary calcium and vitamin D; anabolic and catabolic effects of parathyroid hormone; cellular actions of parathyroid hormone on osteoblast and osteoclast function; autocrine and paracrine functions of parathyroid tissue; and the chemistry and biology of parathyroid hormone secretory protein. In Chapters 8–16, parathyroid hormone is considered with respect to the discovery by Aurbach of one of its second messengers, cAMP; regulation of its biosynthesis and metabolism; the parathyroid hormone gene; structure–function analysis of parathyroid hormone and parathyroid hormone-related protein; measurement of parathyroid hormone in the circulation; parathyroid hormone and parathyroid hormone-related protein as polyhormones; receptors for parathyroid hormone and parathyroid hormone-related protein; G-proteins as transducers of parathyroid hormone action; biochemical mechanisms of parathyroid hormone action. The book proceeds in Chapters 17–20 to a consideration of PTHrP: its structure, physiological processing, and actions; its causative role in hypercalcemia of malignancy; its skeletal and renal actions; and its measurement in the circulation. Other causes of hypercalcemia, besides PTHrP, and the management of PTH and PTHrP-dependent hypercalcemia complete this section (Chapters 21–22).

Section II, Clinical Concepts of the Parathyroids, begins with an 18-chapter section on primary hyperparathyroidism (Chapters 23–40). This segment is a full exploration of the hyperparathyroid state from theoretical aspects of parathyroid cell growth to the molecular basis of primary hyperparathyroidism. A discussion of the spectrum of parathyroid tumors leads to a consideration of its modern clinical presentations and the course of primary hyperparathyroidism. The change in clinical presentation of primary hyperparthyroidism from a disease of bones, stones, and groans to a relatively asymptomatic disorder does not lose sight of a major clinical complication, nephrolithiasis, which is still seen in patients on a regular basis. A chapter devoted to newer markers of bone turnover in primary hyperparathyroidism is followed by a discussion of the histomorphometric features of the disease. Medical and surgical management of primary hyperparathyroidism and the role of preoperative localization techniques are covered completely. Unusual manifestations of primary hyperparathyroidism include separate discussions of parathyroid carcinoma and acute primary hyperparathyroidism. The MEN syndromes I and II focus on the parathyroids, as does the chapter on familial hypocalciuric hypercalcemia. In Chapters 41 and 42, the parathyroids in renal disease are reviewed with respect to pathophysiology, clinical profile, and management.

Chapters 43–47 cover the hypoparathyroid states with respect to differential diagnosis, autoimmune etiologies, molecular genetics, and a special consideration of the clinical, biochemical, and molecular features of pseudohypoparathyroidism. A separate chapter is devoted to the therapy of hypoparathyroidism.

The last four chapters of the book, Chapters 48–51, cover unusual aspects of the parathyroids: parathyroid function in the pathophysiology of osteoporosis and parathyroid hormone as a potential therapy for osteoporosis. Parathyroid functions in Paget's disease of bone and in magnesium deficiency complete the treatise.

We recognize that few readers will read this book from cover to cover, although many of the chapters are closely interrelated. In order to permit virtually all chapters to "stand alone" but also to be connected to the rest of the book, we have liberally included cross-references to other chapters where appropriate. The reader can thus easily refer to other chapters for more information on a given subject. This design also necessarily calls for some interdigitation between chapters so that the reader is not always required to refer to another chapter but, rather, can get a brief summary in the chapter being read of an area that is covered more completely elsewhere.

If it was true that we needed a book on this subject five years ago when the idea was first germinating, why did it take so long to get it done and what was the impetus for finally accomplishing the task? The first of these two questions has a simple answer. Ideas for books are rather easy to develop but it is quite another matter to mobilize an army of over 90 experts to bring that idea to reality. As is true for so many things, this idea was put on the shelf to be admired for its own sake and to be completed later. The mobilizing impetus and the inspiration for this effort eventually did come. Regrettably, it came in the form of a tragic event in our lives, the death of Gerald D. Aurbach.

The death of Jerry on a street in Charlottesville, Virginia, on November 4, 1991, was random, senseless, and violent. At 64 years of age, Jerry was still alive with love for his work, his family, and his friends. In a moment, we suddenly lost a man who guided the very definition of our field for over 30 years. We lost a man who was our teacher and our friend. We lost a brilliant scientist who was involved in most of the major advances in this field over the past three decades. We lost a man who trained an extraordinary number of us for successful careers in basic and clinical investigation of the parathyroids. We lost a gentle man who consistently brought out the best in us. A summary of the many accomplishments that came from Jerry's laboratory and the trainees, collaborators, and associates who worked with him is depicted in the time-line on pages xxvi–xxvii of this book. It is an extraordinary legacy. The two *IN MEMORIA,* by Bilezikian (*Journal of Bone and Mineral Research* 7:ix–x, 1992) and by Potts and Spiegel (*Journal of Clinical Endocrinology and Metabolism* 75:1386–1388, 1992), speak

volumes to his career, to his accomplishments, and to his persona.

In a flash, the dream shelved in the recesses of consciousness and relegated to "when I get to it" became an urgent need. *The Parathyroids* had to be written in the memory and honor of Gerald D. Aurbach, and it seemed altogether fitting that it be written by those who were close to Jerry. We who knew him so well and respected him so much would write a volume for the field. Virtually all of the principal authors of this text fit into that category. Maurice Attie, who also belongs in this book, was tragically killed in a bicycle accident in Philadelphia only a few months after Jerry's death. We remember Maurice and wish that he too were still with us. It is extraordinary that a book designed to be as comprehensive as this could be assembled by a collective authorship whose scientific roots were established by Jerry. His contributions to this field are represented not only by his science but also by his scientific progeny who are the next generation of investigators to study and write about it.

We took up this task with time in mind. *The Parathyroids* had to be published with a short lag time because the book is a timely dedication to Jerry's memory. It had to be published soon because this field is in "fast forward" and if one used the normal publication time for a book of this magnitude, it would run the risk of rapidly becoming outdated. To the credit and thanks to all the authors, virtually all 51 chapters were submitted within a six-month period of time. The dedication of the authors to this task is gratefully acknowledged by us. We also are grateful to Jasna Markovac of Raven Press, who helped to ensure that the process ran as efficiently as possible and whose efforts also were instrumental in ensuring a rapid turnaround time to final publication.

John P. Bilezikian
Robert Marcus
Michael A. Levine

Introduction

A History of the Parathyroid Glands and their Secretory Product, Parathyroid Hormone

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PREHISTORIC DEVELOPMENT

The control of calcium metabolism has been a central evolutionary theme from the very beginning of the primitive life of prokaryocytes almost 4 billion years ago to today's highly specialized life of higher organisms.[1] It is assumed that life on our planet first originated in the sea in the form of primitive unicellular animals that were able to adapt to the special environment that could be found there containing sodium, calcium, and a number of other elements. With its high calcium concentration, however, the sea was not an ideal environment for specialized cellular life. A high calcium ion concentration causes damage to intracellular organelles, aggregates proteins, and nucleic acids, and precipitates phosphates involved in energy transfers—events that would be incompatible with life.[2] In order for primitive cells to handle the poisonous effects of a high calcium environment it was vital to develop mechanisms to maintain very low concentrations of calcium inside cells. The solution to this problem was the development of pumps that could transport the calcium ion across the cell membranes and across membranes of specialized intracellular structures like mitochondria, the Golgi apparatus and the endoplasmic reticulum, which could store calcium inside cells. This created a huge (10,000-fold) calcium concentration gradient (10 mmol/L in the sea vs. 0.001 mmol/L inside the cell). This high transmembrane calcium gradient created ideal conditions for the calcium ion later to be used as a signaling agent.[3]

Primitive animals thus had an internal milieu that accommodated their external saltwater environment. For ocean-dwelling organisms that remained in this environment, a push–pull mechanism for control of internal calcium concentration emerged.[4,5] A pituitary hormone called fish prolactin promotes the ingress of calcium through the gills by increasing the activity of calcium-dependent ATPase (this calciotropic hormone also promotes hypercalcemia in amphibians, reptiles, and birds).[4] This action is counteracted by a peptide hormone called stanniocalcin (STC) (also teleocalcin or hypocalcin), a secretory product of the Corpuscles of Stannius (CS), small endocrine glands located on the ventral kidney surface of bony fish.[5] The primary target organ of STC is the gill, which responds to exogenous STC administration by decreasing calcium transport. An increase in extracellular calcium concentration provides the major stimulus for STC release, whereas removal of CS leads to hypercalcemia, and this is accompanied by a rise in gill calcium transport.[5]

Approximately 400 million years ago certain species were able to leave their marine environment for a life on land. As a result of their developmental history, land animals have an internal milieu that corresponds to their early sea environment. The relative amount of sodium, potassium, and calcium salts in human blood exists in the same proportions as in seawater. Over time the sea has become more saline due to evaporation, and today the amounts of sodium, potassium, and calcium are three times higher in seawater than they are in our blood; however, the relative proportions among the elements are the same. Our ancestors were marine animals, and their legacy to us is the diluted saltwater circulating in our blood.[6]

In terms of developmental biology, parathyroids appeared first in amphibians and these glands were preserved in the animals that later migrated permanently onto land. As a remnant of the prehistoric origin of human beings, the parathyroids originate in the third and fourth branchial clefts of the human fetus and later

The Parathyroids, Third Edition
http://dx.doi.org/10.1016/B978-0-12-397166-1.00064-3

during fetal development they navigate down to their normal position in close proximity to the thyroid.

Although the first appearance of distinct parathyroid glands occurred in amphibia, the ancestry of the parathyroid hormone molecule appears to be much older. The presence of PTH receptors has been confirmed in a range of bony fish.[7] Two PTH genes have been identified in zebrafish and pufferfishes, and their peptide products share moderate sequence homology to human PTH.[8,9] In pufferfish, for example, a peptide has been obtained that shows 56% sequence identity to human PTH(1–34). This molecule, fPTH(1–34) stimulates the cAMP–adenylate cyclase system in cultured mammalian bone cells and also shows anabolic activity on rat skeleton.[10]

Based on internal amino acid sequence and structural homologies of pre-proPTH, Cohn et al. proposed that the mammalian PTH gene arose by reduplication of a primitive gene.[11] In a follow-up to that work, Mallette presented sequence homology evidence that the pre-proPTH gene is the result of a four-fold gene replication.[12,13] Considerable interest has recently focused on possible evolutionary relationships between PTH and PTH-related peptide (PTHrP), a molecule with significant sequence homology to PTH. Most recent work tracks both molecules back in evolutionary time to cartilaginous fish, presumably reflecting their common origin from an earlier precursor molecule (see also Chapters 3 and 5).

FIGURE I.1 Ivar Sandström (1852–1889), Age ≈25 years, taken at the time of his unexpected discovery. *Reproduced with permission from Uppsala University Librarys Archives.*

DISCOVERY OF THE PARATHYROID GLANDS

Knowledge of the existence of parathyroid glands and their physiological role, their disorders, and the mode of action of their secretory product, parathyroid hormone (PTH), has evolved during the last 150 years. Analogous to work in other fields of medicine, the history of parathyroid research is a compelling tale, with steady advances punctuating long periods of stalled progress, angry debates, and missed opportunities.[14,15] We briefly review here the early history of this story, but the reader who wishes more elaborate coverage of that era should consult the comprehensive review by Boothby.[16]

The discovery of the parathyroids was, as were many other discoveries in medicine, a serendipitous event. Although a parathyroid gland was noted by the English anatomist Richard Owen while dissecting an Indian rhinoceros in 1850,[17] credit for the discovery of the parathyroid glands is usually given to the Swedish anatomist and histologist Ivar Sandström (Figure I.1).

Sandström was a 25-year-old medical student working at the Department of Anatomy in Uppsala when, in 1877, he conducted a microscopic examination of the neck area of a dog and came across a structure he did not recognize. He went on to carry out comparative anatomical studies on cats, rabbits, oxen, and horses and found a similar histological picture in all these species. In follow-up, he dissected 50 human corpses in most of which he found two glands on each side of the neck, although their locations, shapes, and color varied among cases[18] (Figure I.2). He gave these newly recognized glands the name we use today: *glandulae parathyroidea.* As an anatomist and histologist Sandström could not go further than to describe the macroscopic and microscopic characteristics of the organ. Naturally enough, he had no clue as to what function, if any, the parathyroids had. However, he assumed that they represented embryonic underdeveloped thyroid tissue and predicted that "later pathologists would find tumors in them."

The Search for a Function

Sandström's discovery published in Swedish received little attention for more than a decade until the French physiologist Eugene Gley, who was studying the thyroid, came across Sandström's report. Gley found that animals sometimes had fatal cramps after thyroid operations and he was first to point out that the parathyroids

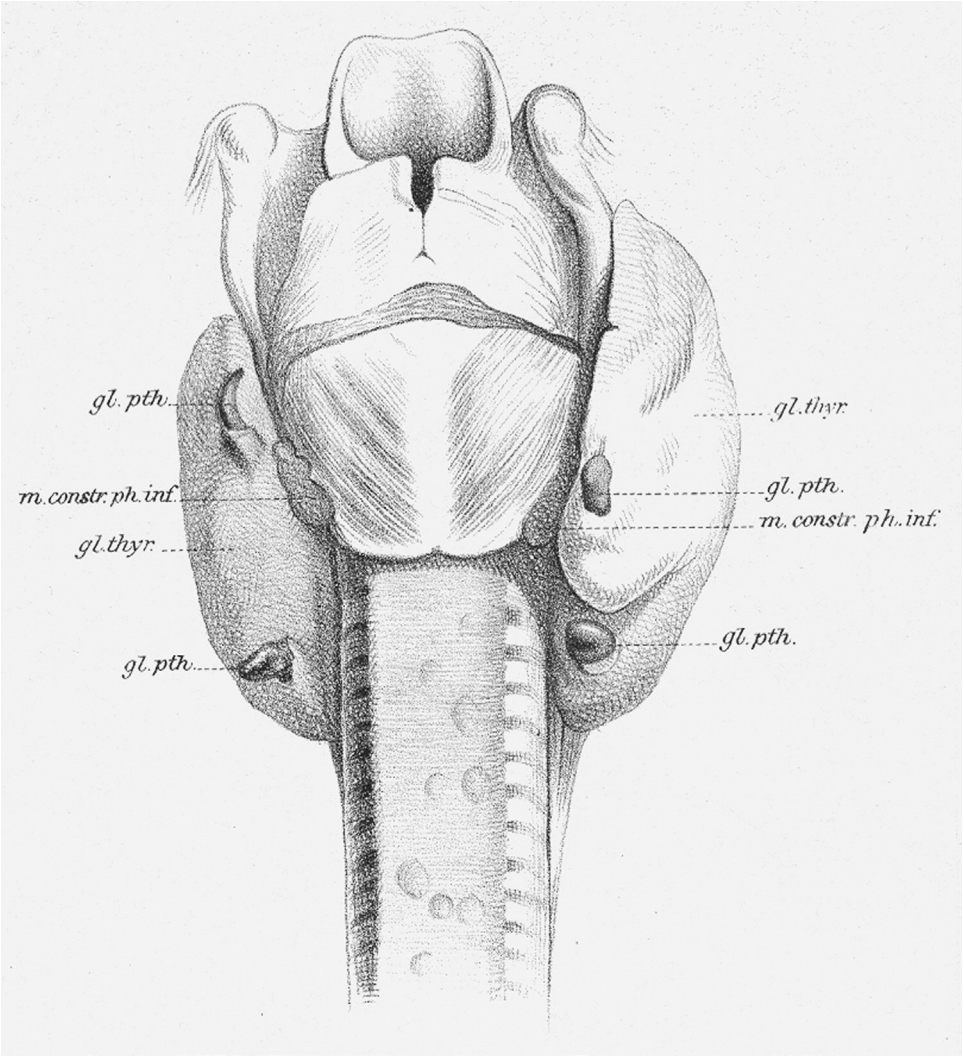

FIGURE I.2 Parathyroid drawing from Sandström's publication in Upsala Läkareförenings Förhandlingar, 1880. *With permission.*

had an important function.[19] Gley's research became the starting point for intense interest in an organ that was obviously vital—at least in experimental animals.

At the end of the 1870s, the noted Viennese surgeon, Theodor Billroth, Professor at Vienna's *Allgemeine Krankenhaus*, one of Europe's most prestigious academic centers, resumed operating on thyroids after having abandoned these operations a decade earlier due to deaths during or soon after surgery of almost half of the patients. One of Billroth's assistants, Anton Wölfler, described an unusual course of events in a patient who 3 days earlier had undergone a thyroid operation. The patient experienced spastic twitching and muscle cramps with the hands in a position characterized as *der Hand des Geburtshelfers* (obstetrician's hand).[20] Of 38 early thyroid operations performed by Billroth, 10 patients experienced postoperative tetany.[21] It is somewhat ironic that Billroth, who had become so well known for his many innovative surgical procedures, experienced a new kind of complication that made way for an entirely new field of research. No one could imagine then that these catastrophic complications would later come to be linked to the minute glands that Sandström was dissecting in Uppsala at the very same time.

In a *Festschrift* published in commemoration of Rudolph Virchow's 70th birthday, the pathologist Friedrich von Recklinghausen described a patient who had an unusual disorder where the calcium content of the skeleton was lower than normal and had been partially replaced by connective tissue and cysts. He named the condition osteitis fibrosa cystica and noted that there was a "small reddish-brown lymph gland on the left side of the thyroid," which fits the description of a pathological parathyroid gland.[22]

Jacob Erdheim, the noted Professor of Pathology in Vienna, was quite aware of the problem with cramps that often occurred after neck operations performed by Billroth and his surgeons. There were different theories for the cause of this phenomenon; some believed it was due

FIGURE I.3 Professor Jacob Erdheim (1874–1937), Austrian pathologist. *Reproduced with permission from Kalmia Press.*

solely to the loss of parathyroid tissue, others thought it was due to removal of the thyroid, and some believed it was a combination of the two. Erdheim carried out the arduous task of performing a complete microscopic examination of the entire neck region of three patients who had died of tetany after thyroid operations, and he could not find residual parathyroid tissue in any of them[23] (Figure I.3). In the laboratory, Erdheim had noted that rats have only one parathyroid gland on each side of the neck and by using a red-hot needle he could destroy varying amounts of parathyroid tissue without injuring the thyroid. When he examined partially parathyroidectomized rats several weeks later he observed discoloration of their front teeth. The incisor teeth of rats are special in that they continue to grow throughout their entire lives. Erdheim was able to establish that calcium could only be deposited in the rat's growing teeth if enough parathyroid tissue had been left in the neck.[24]

Parathyroid Pathology in Early Cases

The emergence and evolution of hyperparathyroidism as an important clinical entity is discussed later in this chapter and is comprehensively described in other chapters of this book. Here we report briefly how the early cases of parathyroid tumor and postoperative tetany led to an understanding of the connection between the parathyroids and calcium.

Several isolated cases of parathyroid tumors were described some 20 years after Sandström's discovery. The first account by DiSanti in 1900 was a case where the tumor was described as having been very large "without having fulfilled the criteria for being a cancer."[25] The first report of an enlarged parathyroid gland that was surgically removed was provided by the Belgian surgeon C. Goris. The patient was a 22-year-old man who was found to have a cystic parathyroid tumor. It is evident from Goris' report that previous parathyroid operations must have been undertaken because he stated, "an enlarged parathyroid gland is certainly not a rarity; however, it is not an ordinary tumor either."[26]

The Parathyroid–Calcium Connection

In the early 1880s, the British physician Sidney Ringer, working at University College Hospital in London, used isolated frog hearts to study the effect of different substances contained in blood on the heart's ability to contract. Ringer was able to show that calcium was essential for contraction. Without calcium the heart soon stopped in a cramped state, "water rigor." Only when calcium was present in the solution could the cardiac chambers expand so that the heart worked normally. Ringer's experiments showed that calcium had hormone-like effects and worked like a "first messenger" to trigger a physiological effect.[27]

The German-American physiologist Jacques Loeb, like Ringer, was interested to understand the effects of different salts on physiological functions. He also found that calcium was imperative to prevent muscle cramps and to sustain a number of other functions. His later research would be more focused on questions about the origin of life and his spectacular findings would make him one of the most renowned American scientists. Using sea urchins, jellyfish, and various other marine animals in his experiments, he showed that by chemically changing the surrounding environment, artificial fertilization of sea urchin eggs could occur that resulted in their developing into normal larvae.[28] Loeb's research proved that calcium and other substances could function as "second messengers" inside the cells by being able to initiate a surge of biological events that could trigger different physiological effects, for example muscle contraction, initiation of cell division, or secretion of a hormone.

The person who first put calcium on the agenda as far as the parathyroids were concerned was a young pathologist at Johns Hopkins University, William MacCallum (Figure I.4). His interest in the parathyroids had been aroused by the surgeon William Halsted, around 1905, and he performed a number of experiments where he gave parathyroid extract to dogs that had had their parathyroids removed. MacCallum and his colleague Carl Voegtlin showed a few years later that the cramps that dogs suffered after having their parathyroids removed

FIGURE I.4 Professor William MacCallum (1874–1944). *Reproduced with permission from the National Library of Medicine; the Chesney Archive of Johns Hopkins University, Baltimore, MD; and the Underwood Archives, Woodside, CA.*

disappeared almost instantly when calcium was injected into the bloodstream. They also reported that the parathyroidectomized dogs had blood calcium concentrations that were approximately less than half of those found in normal dogs.[29]

By around 1910, experiments on frogs, rats, and dogs had, in principle, solved the underlying problem of cramps that appeared after neck surgery. However, there were many who felt that the calcium hypothesis alone could not explain the entire phenomenon. A theory of some kind of autointoxication existed for a long time and many thought that the main function of the parathyroids was to purify the blood of harmful substances. In 1923, the Norweigian physiologist Harald Salvesen published an article where he described his results of a series of experiments in dogs.[30] Salvesen began his article by observing "We have no certain knowledge about the function of the parathyroid." After having presented the results of his carefully executed experiments he drew the following conclusions: "These experiments show that the characteristic feature of parathyroid deficiency is a lowering of the calcium levels in the blood, which is more pronounced when more glandular tissue has been removed. The studies show that the parathyroids control the metabolism of calcium, and in this way not only the function of the muscles and nerves are affected, but the functions of all other organs as well."

Nearly 50 years after Sandström's discovery, the function of the parathyroids had finally been established. At the time, most people thought that they knew everything that there was to know about the parathyroids; certainly they were vital, but if care was taken not to remove them accidentally during neck surgery, they were assumed to have no clinical importance. However, it would soon become apparent that pathological changes in these glands could be the cause of a serious clinical picture. Another half-century later, thousands of patients around the world were diagnosed with primary hyperparathyroidism (HPT).

DISCOVERY OF THE PARATHYROIDS' ACTIVE PRINCIPLE

In the late 1800s, many medical practitioners were offering their patients a variety of diverse extracts made of animal tissue; organotherapy was introduced for treatments using these extracts that were claimed to be effective against almost every and any possible affliction or disease. In 1891, George Murray demonstrated that myxedema could be successfully treated with ovine thyroid extract,[31] but apart from this there were no other examples that organotherapy could cure disease. The French physiologist Gustave Moussu was one of the first to observe that parathyroid extract from horses could remedy cramps following neck surgery in dogs.[32] Some of Halsted's patients at Johns Hopkins who suffered tetany after neck surgery were also treated with parathyroid extract. MacCallum found that the effects on cramps varied: sometimes it worked well, in other cases nothing happened.

The discovery of parathyroid hormone (PTH) initiated a long and bitter dispute over the intellectual rights to the discovery, the patent rights, and the commercial exploitation of parathyroid extract. Adolph Hanson was a surgeon with a private practice working in Faribault, Minnesota, and in 1922 he began chemically analyzing the parathyroids of beef cattle. An amateur chemist who performed unconventional experiments, he discovered that boiling the parathyroids in hydrochloric acid produced an abundant precipitate, which he assumed contained some kind of active substance. Hanson himself had no resources for testing the physiological effects of his extracts so he sent them to researchers working at university laboratories. Despite some encouraging results no conclusive effects of the extracts could be established. Hanson published his results in several articles in *Military Surgeon*,[33] a publication read almost solely by military doctors (Figure I.5).

Two years after Hanson's publication, James B. Collip, an established Canadian university professor, reported that he had succeeded in producing an active hormone from parathyroid extract[34] (Figure I.6). Collip had a great deal of experience in isolating substances from extracts after having being involved with the discovery and isolation of insulin a few years earlier. He used large amounts of tissue, usually 75–100 parathyroid glands from oxen for the preparation of his extract, which was obtained by incubation with hot

FIGURE I.5 Adolph Hanson (1888–1959). Private practice surgeon and amateur chemist, discoverer of parathyroid hormone. *Courtesy of Wangensteen Historical Library, University of Minnesota.*

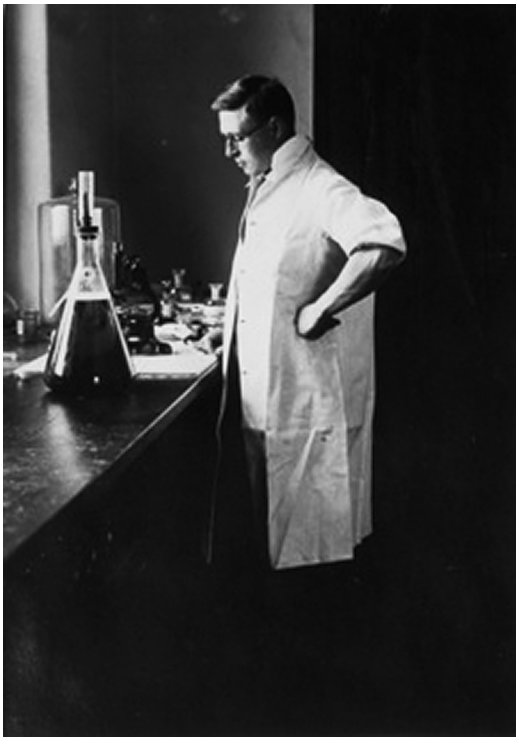

FIGURE I.6 James B. Collip (1892–1965). Canadian chemist. *Courtesy of the Thomas Fisher Rare Book Library, University of Toronto.*

hydrochloric acid. Collip's extract had greater purity and activity than Hanson's, and its physiological effects were reliably more effective for preventing tetany in parathyroidectomized dogs. Collip also successfully treated a boy with tetany with his extract and demonstrated that, in large doses, it caused von Recklinghausen's disease of bone. Collip named the new hormone parathyroid hormone (PTH).

Less than 2 years passed between Collip's first tests and the delivery of a commercial product, Para-Thor-Mone (Eli Lilly), to market. The targeted use for this product was the management of many patients who had developed signs and symptoms of permanent hypoparathyroidism following thyroid surgery for goiter. Hanson accused Collip of not having given him the acknowledgment he felt was his due but Collip denied ever knowing anything about Hanson's early experiments. The bitter dispute over the patent rights that evolved has been reported elsewhere.[6,15,35] Even though Hanson finally was awarded priority in the discovery, Collip's work was more readily accepted by the scientific community because the growing profession of endocrinology preferred to accept the contributions of a biochemistry professor with a grand discovery already to his credit, over that of a small-town practitioner and amateur chemist.[36]

THE SEARCH FOR MECHANISMS OF PTH ACTION

The search for mechanisms has been intense from the early days following the availability of Collip's fairly reliable parathyroid extract with which one could carry out animal experiments. However, this search was not particularly fruitful until the modern era.

Postoperative Tetany

One of the first issues to be addressed was the means by which removal of parathyroid glands produced tetany and by which parathyroid extract could prevent or rapidly treat it. Early causal theories for postoperative tetany have been described above, with opinion divided regarding the roles of calcium deficiency versus "autointoxication." MacCallum and Vogel showed that nervous hyperexcitability could be induced by cross-circulating blood from affected animals into normal animals and found also that injection of a parathyroid extract failed to reduce the level of excitability.[37] They attributed increased excitability to a change in the nature of the perfused blood, possibly a lower calcium concentration, but unfortunately they used an aqueous extract of parathyroid glands that was likely inert, thereby causing them to miss any conceivable preventive or therapeutic effect. Attention quickly turned to the concept that tetany may result from the accumulation of neurotoxins, and

various candidate molecules were proposed, including methyl guanidine, methylcyanamide, phosphates, and carbon dioxide, among others.

Following the finding of methyl guanidine in the urine by Koch in 1912[38] and the subsequent discovery by Paton et al. that both guanidine and methyl guanidine cause tetany in rats, a series of papers appeared aiming to validate intoxication by guanidine compounds as the cause of tetany in PTX animals (summarized in reference[39]). It was reported that in the absence of PTH, dimethyl guanidine is produced from creatinine and somehow reduces ionized calcium activity in blood.[40] Given the similarity of PTX-associated symptoms to those of guanidine poisoning, the view gained traction for several years that PTH acts as an important regulator of guanidine metabolism.

The guanidine accumulation hypothesis lost support, however, when Collip and Clark induced fatal tetany in dogs by methyl guanidine administration, despite concomitant administration of their acid extract of parathyroid glands, which was attended by an elevation in blood calcium.[41] Moreover, it subsequently became evident that apparent increases in circulating guanidine derivatives were based on faulty methodologies.[42] Final proof of the hypothesis that PTX-related tetany is related to hypocalcemia was shown by Collip, who both prevented and treated tetany in PTX dogs by administration of parathyroid extract sufficient to raise blood calcium[43] (Figures I.7a and I.7b).

PTH Action: Renal or Skeletal?

A second area of active inquiry was whether the effects of PTH were exerted directly on the skeleton, or whether they were secondary to actions on other tissues, primarily the kidney. In particular, Dr. Reed Ellsworth, working initially with Fuller Albright and later independently, made important contributions to the understanding of the renal effects of PTH. In the fifth of a series of papers on this topic, Ellsworth, in 1932, proposed the following rationale for his views:[1] PTH lack results in a fall of urinary phosphorus, a rise in serum phosphorus, and a fall in serum and urinary calcium whereas administration of PTH corrects these abnormalities.[2] Given substantial evidence that calcium and phosphorus are closely interrelated, "it seems probable" that PTH affects one of the changes mentioned above, the others being sequelae.[3] Because the rise in urinary calcium is much delayed compared to onset of phosphaturia, which is immediate, it appears that phosphorus changes are primary.[44] It is interesting to note that nowhere in his series of papers did Ellsworth entertain the possibility that PTH could exert two independent actions, one on calcium and the other on phosphorus. In addition, it must be noted that relative changes in circulating calcium and phosphorus in response to PTH are highly species- and circumstance-dependent. Rats, for example, show large increases in calcium, but do not show corresponding increases in inorganic phosphorus, whereas in dogs PTH increases both calcium and phosphorus. Further, combined infusion of glucose and insulin leads to a drop in plasma inorganic phosphorus concentration, but calcium concentration does not rise, whereas administration of

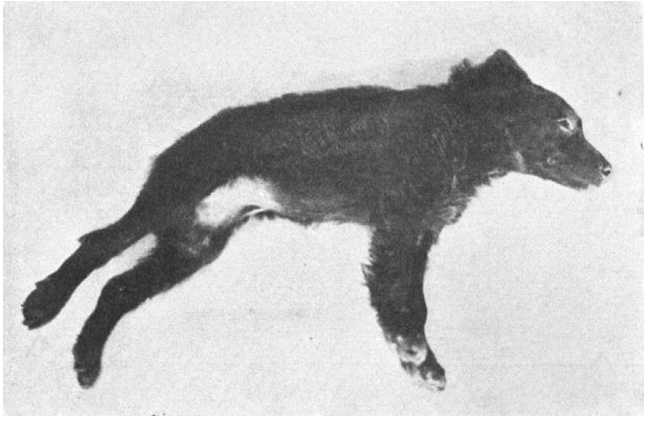

FIGURE I.7A Experimental dog 59 days following thyroparathyroidectomy. The animal is in a state of severe tetanic contraction.[41] *Reproduced with permission from the Journal of Biological Chemistry.*

FIGURE I.7B Experimental dog shown in Figure I.7a 3 hours following subcutaneous injection of parathyroid extract. The animal is normal.[41] *Reproduced with permission from the Journal of Biological Chemistry.*

l-thyroxine leads promptly to increases in both calcium and phosphorus.

On the other hand, substantial evidence emerged during this same time frame for a direct effect of PTH on calcium homeostasis. Thomson and Pugsley produced hypercalcemia in dogs with no concomitant fall in plasma phosphorus,[45] and in a follow-up study from the same laboratory, nephrectomized rats administered PTH for 2 days showed marked signs of osteoclastic bone resorption, leading to the conclusion that "the effect of PTH on bone is independent of any direct influence it may have on the renal threshold for phosphates."[46]

One additional point concerning the early studies of PTH action using the Collip extract concerns its anabolic effect on bone. Evidence for such an effect was suggested in studies by Bauer, Aub, and Albright in rats showing an increased trabecular number following administration of the extract,[47] and a study by the Hungarian-Canadian physiologist, Hans Selye, who reported that Parathyroid Extract showed anabolic properties on the rat skeleton.[48] These findings in animals, which presaged later development of PTH as a treatment for osteoporosis, were largely ignored for the next 50 years.

As it turned out, significant further progress in understanding the mechanism of PTH action would await new approaches to purifying and characterizing this hormone. Although the Collip extract contained several active constituents, its long-term stability was not reliable. Moreover, his HCl-extraction method created a large number of inert peptide fragments capable of promoting the formation of neutralizing antibodies that attenuated therapeutic efficacy of the extract. It is now understood that PTH contains several aspartate residues, which are sites for acid protein hydrolysis.[49] The HCl-acid extraction technique created so many peptide fragments that attempts at further hormone purification were stymied.[50]

Thus, long-term success in managing hypoparathyroidism with Parathyroid Extract was not achieved. In the face of these issues, the introduction of vitamin D synthesized by UV irradiation of plants offered an inexpensive approach, along with calcium supplementation, to managing patients with hypoparathyroidism.[51,52] Parathyroid Extract-Lilly continued to be marketed well into the 1970s and beyond, but its use became confined to the classic diagnostic test introduced by Ellsworth and Howard[53] to distinguish patients with idiopathic or surgical hypoparathyroidism from those with pseudohypoparathyroidism.

MODERN APPROACHES TO PTH STRUCTURE AND FUNCTION

In 1959, Dr. Gerald Aurbach (to whose memory this book is dedicated), while a post-doctoral fellow in the laboratory of Edwin B. Astwood at Tufts University, reported that extraction of bovine parathyroid glands in phenol yielded a biologically active preparation of reasonable potency.[55] Aurbach reasoned that although heated acidic solvents released active peptide from glandular tissue, it also cleaved the peptide. He reasoned that phenol as a strong organic solvent could also free the hormone from glandular constituents but without damaging the presumed peptide. On precipitation in trichloroacetic acid, the phenol extract gave a stable product that could be purified to homogeneity with countercurrent partition chromatography. The success of this approach constituted a major breakthrough that allowed the hormone to be purified in sufficient bulk to embark on a systematic exploration of its nature, its amino acid sequence in human and several animal species, its structural characteristics, and its structure–function relationships (see "Resolution of parathyroid hormone structure," below).

Before leaving the substance of Aurbach's original paper, it is of interest to quote here an additional point that he addressed and which directly relates to earlier speculations about the mechanism of PTH action: "Early crude extracts which had marked effects on calcium metabolism also enhanced the excretion of phosphate in the urine. Other investigators had proposed that a distinct hormone may account for the latter effect. The isolation of a single substance, a potent mediator of both biological effects, seems to end this controversy."[55]

Approaching the Mechanism of PTH Action

Subsequent work from Aurbach's laboratory was conducted at the National Institutes of Health (NIH) in collaboration with Dr. John Potts, who was initially at NIH but moved later to the Massachusetts General Hospital. While this scientific collaboration, as well as those in other laboratories, was intent on defining the amino acid sequence of PTH, other work during the 1960s was focused on mechanism of action. A number of false starts did not prove fruitful. In this era, attention was given to so-called "allosteric" effects of hormones, by which was meant the binding of hormones directly to intracellular proteins, thereby altering their fine structure and function.[56] Although evidence for such effects was reported for a number of steroid hormones,[57] no real evidence for a similar mechanism ever developed for peptides. Another false start was the observation that active PTH peptides altered various aspects of mitochondrial function, including ion transport, respiration, and ATPase regulation.[58–61] Initially, it appeared that these mitochondrial effects were fairly specific for PTH and not replicated by non-parathyroid peptides. Interest in this line of inquiry diminished when, in 1965, Aurbach and colleagues reported that similar actions could be achieved with two biologically inert peptide fractions

from parathyroid glands as well as various basic proteins, such as protamine and polylysine.[62]

In that same year, Aurbach attended a seminar by Professor Earl Sutherland on the discovery of 3′,5′-cyclic adenosine monophosphate (cAMP), its enzymatic synthesis by the membrane-bound enzyme adenylate cyclase, and its role as a "second messenger" in the activity of catecholamines. Impressed by Sutherland's comment that activation of this system led to a prompt rise in the urinary excretion of cAMP, Aurbach and his collaborators were able to demonstrate that PTH stimulated the accumulation of cAMP in rat calvaria and renal cortical tissue, and that this action came about by direct stimulation of adenylate cyclase in those tissues.[63–65] They also demonstrated that infusion of PTH into humans led to a prompt rise in urinary cAMP excretion that preceded the classic phosphaturic response, a phenomenon that was severely blunted in patients with pseudohypoparathyroidism.[66,67]

Subsequent to these developments, the adenylate cyclase–cAMP system received wide acceptance as an important "second-messenger" system for PTH action, a view that has become a dominant thread in considerations of PTH actions. However, it is also the case that the last 40 years of research has broadened substantially the list of candidate systems that may be activated by PTH. These include direct stimulation of calcium ion flux in bone cells,[68–71] activation of the phosphoinositol pathway,[72–75] and, most recently, activation of the Wnt signaling pathway.[76] Further discussion of these fascinating developments exceeds the scope of this historical review, but these will be considered elsewhere in this book.

Resolution of Parathyroid Hormone Structure

The series of experiments concerning the cAMP system described above led to another development in the Aurbach laboratory that greatly aided exploring the structure–activity relationships of PTH, the validation of a reliable bioassay *in vitro* for hormonally active parathyroid peptides. Until then, bioassay of parathyroid activity was conducted by measuring the rise in blood calcium in thyroparathyroidectomized animals. Such an assay was time consuming and costly in animal resources. In 1969, Marcus and Aurbach reported that homogenizing rat renal cortices in 10% dimethylsulfoxide resulted in a membrane preparation that was stable to long-term storage in liquid nitrogen and showed highly sensitive, specific, and reproducible stimulation of the adenylate–cAMP system by PTH.[77] This assay proved invaluable in subsequent evaluation of the biologic potency of a number of parathyroid peptides that were synthesized in the course of studies aimed at resolving the full amino acid sequence of PTH.

Resolution of the PTH structure involved a collaborative multi-disciplinary effort of the Aurbach and Potts laboratories, requiring expertise in both peptide sequencing and solid-state synthesis, provided by Drs. Hugh Niall and Geoffrey Tregear, respectively. It must be stated that this collaboration was not completely alone in this effort, as the laboratory of H. Bryan Brewer (a former post-doctoral fellow with Dr. Potts) was independently working on the same problem. Using the sequential degradation technique of Edman, both groups reported almost simultaneously in 1970 the amino acid sequence of bovine PTH,[78,79] followed subsequently by similar reports for the porcine[80] and human hormones.[81,82] Several discrepancies in structure were found between the two laboratory groups, but in a series of elegant experiments including nucleotide sequence analysis of the coding portions of the PTH gene, the structures reported by the Potts group turned out to be correct.[83,84] No obvious explanation for the discrepant sequences has been reported. One hypothesis held that the original parathyroid material studied by one of the two research groups may have contained a minor variant, or isohormone of PTH, but no evidence for this possibility has emerged. Thus, the synthetic peptides used today in parathyroid research are based on the hormone sequences originally reported and validated by the Potts group.

Beyond resolving the amino acid sequence of these hormones, the Potts group synthesized and characterized a large number of peptides of varying length and amino acid sequence. The results of this work indicated that the biologically active portion of PTH resides in the amino-terminal portion of the molecule, and suggested that a peptide consisting of the 1–29 amino acid sequence could support the known actions of the full 1–84 PTH sequence.[85] This amino-terminal peptide increases blood calcium in rats, promptly stimulates the urinary excretion of cAMP and phosphate, and is a potent activator of cAMP production in bone and kidney.

This line of structure–function analysis, conducted largely with the bovine hormone, led to the following conclusions regarding PTH action: peptides of approximately 34–38 amino acids from the amino-terminus are fully active *in vitro*, and have *in vivo* potencies approaching those of the intact PTH(1–84). Truncation of these peptides from the carboxyl-terminus to lengths of 27 amino acids or less seriously attenuates biological activity. By contrast, the effect of deleting the first and second amino acids from the amino-terminus is far more profound. Despite binding to the PTH receptor with full avidity, a peptide in which the first amino acid has been deleted loses substantial activity, and elimination of the first two amino acids results in complete loss of biological activity.[86]

Periodic reports have suggested biologic relevance for epitopes in the carboxyl-terminal region of PTH. For example, it was proposed that a distinct alkaline phosphatase activity is stimulated by carboxyl-terminal sequences.[87] The work of Divieti, Bringhurst, and colleagues indicated the presence of receptors for

carboxyl-terminal PTH fragments on bone cells[88,89] but attempts to clone such a receptor have not been successful.

As a consequence of the structure–function research discussed above, it became feasible to synthesize large quantities of parathyroid peptide in the laboratory for use in additional studies *in vivo*. For this purpose, hPTH(1–34) (teriparatide) was selected.[90] One occasionally hears statements that teriparatide is an important physiologic metabolite of the parent intact molecule. Those statements are incorrect. It is true that cleavage of intact PTH in hepatic Kupffer cells generates multiple peptide fragments of variable activity and some hPTH(1–34) might conceivably be produced by this means, but it normally cannot be detected in the circulation. The prominence of teriparatide in parathyroid research reflects the fact that solid-state peptide synthesis, which is highly faithful up to peptide lengths of about 30–35 amino acids, was, at that time, confounded by degeneracy during synthesis of larger molecules. Therefore, because the properties of teriparatide were sufficient to support the known actions of PTH, and because it could be synthesized with great accuracy, this peptide was chosen for large-scale production. Once the structures of parathyroid peptides were reported, a number of commercial organizations undertook the solid-state synthesis and commercial manufacture of teriparatide, which was sold to investigators worldwide for at least two decades, leading to an explosion of interest and ferment in parathyroid hormone physiology.

PRIMARY HYPERPARATHYROIDISM—A NEW DISEASE

There had been isolated cases with osteitis fibrosa cystica in the literature after von Recklingshausen's first report in 1891, but its cause and relationship with the parathyroids was unknown. The first operation on the parathyroids for osteitis fibrosa cystica was performed by the Austrian surgeon Felix Mandl in Vienna in 1925.[91] The patient, a 38-year-old Viennese tram driver known as "Albert J," had a 5-year history of suffering from fatigue and skeletal pain. Radiographs had shown a typical picture of osteitis fibrosa cystica. On admission to hospital he was unable to walk or even stand up, he was emaciated and he had kidney stones. Attempts had been made to treat him with mercury, cod liver oil, electric treatments, and mud baths. Thyroid and parathyroid extracts had been administered without effect. There was a strong suspicion that a parathyroid disorder was involved, but was it hypo- or hyperfunction? Four parathyroid glands were taken from a patient who had died from trauma and transplanted to Albert J, but the transplant did not improve the patient's condition. Mandl considered that if the cause was not parathyroid *hypo*function it could perhaps be *hyper*function. Therefore, he performed an operation on Albert J under local anesthesia and soon found and removed a yellowish brown 25 × 15 × 12 mm tumor. The effect of the operation was dramatic and of almost biblical proportions. The patient's urine was completely clear and the calcium concentration in the urine sank to less than one-fifth of what it had been before the operation. He gained weight, and after a while he could stand and walk using a cane. Radiographs a few months later showed considerable improvement in skeletal density.

As a result of the operation, important insights were gained into the cause of this disease and its clinical consequences: the skeletal changes in von Recklinghausen's disease were due to a parathyroid tumor and not *vice versa* as the advocates of the compensation theory had believed. The successful outcome of the operation soon became widely recognized among surgeons in Europe, resulting in many surgeons operating on parathyroids. The name for the disease, hyperparathyroidism, was coined by one of Mandl's colleagues, E. Gold, after having operated on another patient with a similar clinical picture as Albert J's.[92]

Early Metabolic Studies in Hyperparathyroidism

On the other side of the Atlantic, another patient, Charles Martell, fell ill with a clinical picture typical of osteitis fibrosa cystica. The course of Martell's illness would be even more complicated than Albert J's. His metabolism would be scrutinized by some of the most prominent researchers and endocrinologists of the times, he would undergo a total of seven neck operations, and his disease would be described in the most prestigious medical journals, including three separate articles with a total of 30 pages in the *Journal of Clinical Investigation.* Martell initially came under study by Eugene DuBois in New York who found him to be hypercalcemic and osteopenic.[93] Since Collip's studies had shown that the administration of large amounts of parathyroid extract could induce hypercalcemia it was suspected that Martell's illness was due to an overproduction of PTH. Without knowledge of the successful operation on Albert J in Vienna almost 1 year earlier, a neck exploration was performed but no enlarged parathyroid glands were found. After a second negative operation had been performed Martell was referred to Joseph Aub in Boston for a more detailed study of his calcium metabolism.

Fuller Albright was at the time a young medical student working in Aub's laboratory (Figure I.8). He had recently returned to Harvard after having spent a year as a visiting researcher with Jacob Erdheim in Vienna. Albright studied the effects of PTH on calcium metabolism and he was particularly interested in the effects of the hormone on the composition and function of the

FIGURE I.8 Fuller Albright (1900–1969). American endocrinologist. *Reproduced with permission from Kalmia Press.*

skeleton. Albright found that Martell had a negative calcium balance and that Martell's metabolism resembled that of a healthy person who had been given 100 units of Collip's extract.[94] He concluded that Martell had PTH overproduction, that is, he suffered from a hyperactive parathyroid tumor. Consequently, Martell underwent several additional operations, all with negative results. Finally, after more than 5 years of intense metabolic study, a new operation was performed after a report appeared of an intra-thoracic location of a parathyroid tumor. At this seventh operation on Martell a greatly enlarged parathyroid tumor was found and removed. The operation was successful at last, but unfortunately too late, for Martell died a few weeks later from kidney infection and renal failure.

In the beginning of the 1930s, there were many reports of enlarged parathyroid tumors that had been successfully treated both in Europe and the United States. HPT as a particular disease entity had become well established almost half a century after Sandström's discovery and his prediction that tumors would be discovered in the organ had proven true. In addition, it had been established that the parathyroids were part of the endocrine system, and that HPT was a disease with chemical, organ-specific, and clinical characteristics. However, the full clinical picture of the disease and its pathophysiological characteristics were far from being completely understood. Although the curative nature of surgery was well established, faulty diagnoses and unsuccessful operations were not unusual. On the whole, it was still rather unclear as to what kind of disease HPT really was.

The Emergence of Primary Hyperparathyroidism as an Important Clinical Entity

The many facets of primary hyperparathyroidism (HPT), its epidemiology, clinical presentations, physiology, diagnosis, and therapeutics, are comprehensively discussed elsewhere in this volume and will not be detailed in this chapter. However, it is relevant to remind the reader that progress in the understanding of basic parathyroid structure and function that are under consideration here came about during the 1960s and 1970s, in which tremendous interest in HPT simultaneously emerged. Two important developments led to this emergence. The first was the introduction of routine multiphasic chemistry screening in clinical laboratories, leading to the discovery, particularly in postmenopausal women, of a much higher than expected prevalence of hypercalcemia as reviewed by Heath.[95] The second factor was the development and refinement of radioimmunoassays for PTH,[96] making it possible to validate a given patient's hypercalcemia as due to an increased circulating concentration of the hormone. These are briefly discussed below.

Primary Hyperparathyroidism is a Common, Frequently Asymptomatic Disease Entity

In September 1933, an editorial in *The Lancet* read:[97]

> Hyperparathyroidism is a conception less than eight years old, and there can be few maladies, old or new, whose mystery has been solved so speedily. A decade ago we had no proof of the relationship between parathyroid activity and the metabolism of phosphorus and calcium, and the advance of knowledge since that time shows medical research *at* its happiest. The cooperation of medicine, dietetics, experimental pathology, histology, physiology, and chemistry has directed the surgeon's knife towards a tumour he could neither see nor feel, and the number of recorded cases of generalized *osteitis fibrosa cystica* which have been arrested or cured by operation is now at least 52. The terrible and crippling *symptoms* caused by generalized *osteitis fibrosa* will soon be a thing of the past.

Despite remarkable advances in the understanding of classical HPT, a new disease entity later evolved—mild, often asymptomatic HPT as reviewed in.[95] This awareness especially developed after the introduction of autoanalyzers that facilitated rapid biochemical screening, resulting in a dramatic increase in the number of individuals diagnosed with HPT. As a result, the clinical picture changed noticeably after 1970 compared to

1930–1970. During the earlier period the patients had more specific and serious symptoms involving the skeleton, kidney stones, higher serum calcium concentrations, and lower serum phosphate levels indicative of a higher degree of hormonal activity. The patients were also younger, and parathyroid tumors were larger during the earlier period.

These important changes in the prevalence and clinical nature of HPT brought new therapeutic challenges, particularly for those patients deemed "asymptomatic." These challenges, as well as subsequent changes in the apparent incidence of this disorder, are fully discussed elsewhere in this volume and will not be considered further in this chapter.

Measurement of Circulating PTH by Radioimmunoassay

Although rapidly adopted and frequently used, PTH immunoassay was plagued with difficulties from its outset. Peptides used initially to generate antibody responses were impure extracts of (largely bovine) parathyroid glands. As such, they contained many carboxyl-terminal fragments that were highly immunogenic but minimally active. As excretion of carboxyl fragments was through the kidney, any degree of renal insufficiency led to fragment retention and artifactual elevation of "PTH" concentrations. The initial wave of PTH immunoassays possessed neither adequate sensitivity nor specificity to reliably measure biologically active circulating hormone in normal individuals. Moreover, the presence of immunogenic fragments distorted the shape of isotope displacement curves so that the curves deviated from parallelism with "true" PTH. This appeared to create particular difficulty in differentiating patients with HPT from those with hypercalcemia of malignant disease. Substantial progress toward rectifying these problems could be achieved only with the simultaneous progress, detailed above, in resolving the sequence and structure–function characteristics of PTH. For example, this body of work led to the widespread availability of pure synthetic peptides for immunizing animals and using as ligands in assay systems. One such antibody, developed to a peptide that had both amino- and carboxyl-terminal components of PTH, the so-called "mid-molecule" region, saw wide use in physiologic studies of HPT as well as being commercially available as a diagnostic aid.[98] Another antiserum, developed to a synthetic 1–34 hPTH peptide, permitted specific determination of blood concentrations of hPTH(1–34) when it was infused in physiologic experiments.[99]

During the last two decades, the so-called "intact PTH" assay, developed against pure 1–84 hPTH, has become the clinical standard for reliable clinical determination of circulating PTH.[100] Finally, it has been possible, using antisera directed against specific components of the PTH molecule, to identify a large circulating 7–84 PTH fragment, which may actually block PTH action at its receptor.[101] Known as "Whole" PTH, this assay system provides accurate measurement of PTH in situations attended by retention of the 7–84 fragment, such as renal failure[102] (see also Chapter 16).

Recognition of Variant Forms of Hyperparathyroidism

The great majority of patients with hyperparathyroidism prove to be sporadic. However, as familiarity with this condition increased and diagnostic testing and screening advanced, an assortment of familial syndromes emerged whose characteristics include parathyroid hyperfunction, due either to adenomas or hyperplasia. These include the multiple endocrine neoplasia (MEN types I and II) syndromes,[103,104] a familial condition of cystic parathyroid adenomatosis,[105] and a syndrome of hereditary hyperparathyroidism with multiple ossifying jaw fibromas,[106] among others. These entities will be detailed elsewhere in this volume (see Chapter 23). Here we describe the condition of familial hypocalciuric hypercalcemia (FHH), known also as familial benign hypercalcemia (FBH), as investigation of this entity led to the seminal discovery of the calcium-sensing receptor (CaSR), which completed the link between circulating calcium and regulation of PTH secretion (for comprehensive discussion of this condition, see Chapter 24).

Briefly summarized, in 1967, Jackson and Boonstra reported on features of hyperparathyroidism in multiple kindreds, one of which appeared to be aberrant in that afflicted family members showed hypercalcemia but no evidence of multiple endocrine involvement. Moreover, affected members were generally asymptomatic and did not benefit from parathyroid surgery. The authors suggested that this unusual condition could represent a new diagnostic entity.[107,108] Several years later, Foley et al. described a 7-year-old boy found to be hypercalcemic during an evaluation for headache. Eleven members of his family, extending back several generations, were shown to be hypercalcemic, although none apparently had symptomatic consequences. The boy underwent parathyroidectomy but remained hypercalcemic. Foley et al. considered this to be a distinct syndrome, which they named familial benign hypercalcemia.[109] Over time, as a number of cases of apparent surgical failures for primary hyperparathyroidism became referred to major centers, such as the National Institutes of Health (NIH) and the Mayo Clinic, large series of FBH kindreds were accumulated.[110,111] In the NIH series of 15 kindreds, affected family members experienced

more symptoms than non-affected members, and these were often non-specific manifestations, such as fatigue, weakness, and "mental problems."[110] By contrast, in the Mayo Clinic series of 21 kindreds, where the index case has been excluded, no difference could be shown in the degree or types of symptoms reported by affected versus non-affected members; affected individuals were generally in good health, their bone mineral density (BMD) was normal, and fractures were not more frequent than expected.[111] With respect to biochemical abnormalities, the dominant feature of FBH is a relatively low level of urinary calcium excretion relative to the serum calcium concentration. The degree of hypercalcemia is highly variable, as are circulating PTH values. Although FBH appears to be a completely benign disorder in adults, an exception to that rule was the case of infants homozygous for this condition, who experienced profound and life-threatening hypercalcemia.[112]

Genetic analysis of FHH kindreds clearly demonstrated an autosomal mode of inheritance,[110,111] but the specific genetic defect remained obscure until 1993, when Brown et al. successfully cloned and characterized a cell-surface CaSR from bovine parathyroid.[113] The large extracellular domain of this 120K receptor contains many acidic amino acid residues, which may participate in calcium binding, and hence in parathyroid regulation by calcium. The CaSR demonstrates similarities to metabotropic glutamate receptors and couples to a seven membrane-spanning domain like those in other G-protein coupled receptors[113] (see also Chapter 24). This receptor is expressed not only in parathyroids, but also in other tissues, such as thyroid C cells, kidney, and brain. That same year Pollak, Brown et al. demonstrated loss-of-function missense mutations of the CaSR in several patients with FHH, thereby establishing that mutations in the receptor are the underlying basis for this disease.[114] Subsequent work has expanded the list of CaSR mutations associated with FHH and has confirmed its role in neonatal hypercalcemia.[115]

HUMORAL HYPERCALCEMIA OF MALIGNANCY AND THE DISCOVERY OF PARATHYROID HORMONE-RELATED PROTEIN (PTHrP)

The notion that PTH could be synthesized and secreted into the circulation by cells other than the parathyroids was first considered by Fuller Albright in 1941 in connection with a hypercalcemic patient with renal cell carcinoma.[116] This concept, referred to as "ectopic production of PTH," was widely accepted by the endocrine community for several decades to explain the occurrence of hypercalcemia in patients with malignant or inflammatory conditions who appeared not to have either bony metastasis or true parathyroid gland hypersecretion. The basis of hypercalcemia in several lymphoid tumors and inflammatory conditions, including sarcoidosis, was shown eventually to be the extra-renal production of calcitriol, the active form of vitamin D,[117,118] whereas hypercalcemic individuals with various solid tumors that were not complicated by skeletal metastasis were considered to have "humoral hypercalcemia of malignancy" (HHM). The initial wave of radioimmunoassays for PTH appeared to confirm that hypercalcemic patients with HHM had elevated circulating PTH concentrations.[119] However, closer scrutiny revealed that the PTH-like substances detected by immunoassay in plasma from cancer patients was distinct from PTH. For example, radioimmunoassay curves using material from cancer patients failed to show parallel displacement to that achieved with true PTH,[120,121] and messenger RNA for PTH was not present in non-parathyroid tumors associated with hypercalcemia.[122] Thus, it began to appear certain that the cause of HHM was not PTH but a separate molecule sharing many of the actions of PTH.[123] Although detailing the train of evidence leading to the discovery of PTHrP exceeds the scope of this chapter, the interested reader is referred to Chapter 3 for a comprehensive description. Suffice it to say that an elegant series of experiments led to the almost-simultaneous reporting of the structure of PTHrP and its gene by three different research groups in the late 1980s.[124–126]

REDISCOVERY OF THE ANABOLIC EFFECT OF PTH

In the early 1970s, the scientific community reawakened to the older descriptions of Parathyroid Extract's anabolic effect. To a large degree, credit for this rediscovery was due to an English pharmacologist, Dr. John Parsons. In a series of investigations, Parsons and his collaborators demonstrated that intravenous injection of PTH resulted in a rapid drop in plasma calcium concentration that seemed most likely to represent an increase in calcium uptake into bone. Although this effect was initially attributed to possible contamination by calcitonin of the PTH preparation, Parsons, Neer, and Potts showed conclusively that it could be elicited by calcitonin-free PTH in dogs.[127] Parsons, an astute reader of early literature, considered it likely that this rapid calcemic response could support the idea that PTH is an anabolic agent for bone. Indeed, in a 1972 review, Parsons and Potts expanded on this idea to sugest that "chronic administration of PTH together with calcitonin would be worth testing in osteoporosis."[128] In a subsequent paper, Parsons and Reitt went